

In Vitro Elution of Daptomycin by a Synthetic Crystalline Semihydrate Form of Calcium Sulfate, Stimulan[∇]

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A synthetic crystalline semihydrate form of calcium sulfate, Stimulan, was evaluated as a biodegradable carrier for the daily in vitro elution of daptomycin. Daptomycin and Stimulan were admixed at a ratio of 95:5. Elution lasted for 28 days. Eluted concentrations peaked on days 1 and 11, when the mean values were 1,320.1 and 949.2 µg/ml, respectively. The lowest eluted concentration was detected on day 28. These results support the application of the system described in experimental models of osteomyelitis.

Chronic osteomyelitis is an infection difficult to treat due both to multidrug resistance of common pathogens and to poor penetration of antibiotics into bone (16). Carriers for local delivery of antimicrobials have been developed, attempting to provide locally high concentrations of antibiotics (5). Some newly developed biodegradable carriers have been shown to be very potent for the eradication of experimental osteomyelitis (6, 7). The semihydrate form of calcium sulfate (CaSO₄), commonly known as plaster of Paris, may be applied as a biodegradable system for local drug delivery. It has been used for decades to fill bone cavities resulting from disease, trauma, or surgery (11). It was recently shown to be potent in vitro for the release of vancomycin, teicoplanin, gentamicin, and clindamycin (15). The antimicrobial applied in such an elution system should be active against the most commonly involved pathogens of chronic bone infections, namely, methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (CoNS) (5). Daptomycin, which is a novel lipopeptide antimicrobial with excellent in vitro activity against these isolates (4), may be a candidate for application in a system for local drug delivery.

Stimulan (Biocomposites, Keele Science Park, Staffordshire, United Kingdom) is a synthetic biocompatible bone graft material made of calcium sulfate. It is completely reabsorbed and replaced with new bone. It is synthesized at 100% purity with no traces of potentially toxic impurities that have been associated with naturally occurring mineral sources of calcium sulfate. It has been recently shown by our group that moxifloxacin and fusidic acid may be eluted at high concentrations in vitro by using Stimulan as a carrier (11). The purpose of the present study was to develop an in vitro system for daptomycin elution by using Stimulan as a delivery system.

Stimulan was mixed with daptomycin (Novartis, Basel, Switzerland) at a ratio 95:5 to a total weight of 3 g. This was put into sterile vials (160 by 100 mm) and left at room temperature for 15 to 30 min for solidification. Five similar vials were

prepared. One milliliter of Mueller-Hinton broth (Trec Diagnostic Systems, West Sussex, United Kingdom) was added over the free surface of each mixture and replaced every 24 h. The vials were incubated at 37°C. On each consecutive day, the eluent was removed, transferred to a sterile plastic tube, and replaced with 1 ml of broth. That procedure was repeated until the optical degradation of the prepared mixture. Samples were stored at –70°C until analysis.

Concentrations of daptomycin were estimated in duplicate by a modification of the method already described (3). Briefly, 300 µl of sample was mixed with 20 µl of 99% methanol and centrifuged for 10 min at 5,000 rpm and 4°C. Twenty microliters of the supernatant was injected into a high-pressure liquid chromatography system (1100 series; Agilent, Waldbronn, Germany) with the following elution characteristics: a Zorbax Eclipse XDB-C₈ (4.6 by 150 mm, 5 µm) separating column (Agilent Technologies) warmed at 37°C, a mobile phase of 32.6% acetonitrile and 67.4% 0.5% ammonium phosphate buffer at a flow rate of 1.5 ml/min, and UV detection at 214 nm. The retention time of daptomycin was 3.3 min, and it was estimated as micrograms per milliliter by a standard curve created with known concentrations of daptomycin. The lower detection limit was 6.25 µg/ml, and the interday coefficient of variation of the assay was 4.7%. Results were expressed as means ± standard errors. The area under curve (AUC) for each vial was determined by the linear trapezoidal rule.

Elution of daptomycin lasted 28 days. Then the prepared mixture was destroyed. Eluted concentrations peaked on days 1 and 11, when the mean concentrations were 1,320.1 and 949.2 µg/ml, respectively. The lowest eluted concentration was detected on day 28, and the mean value was 233.9 µg/ml (Fig. 1). This gradual step-down rate of release over the 28 days was consistent for all five replicates. The mean ± standard deviation AUC of daptomycin elution over this 28-day period was 14,542.7 ± 1,925.9 µg · day/ml. Short peaks of daptomycin release were apparent on days 5 and 11. These may be related to the properties of Stimulan.

Daptomycin is a new lipopeptide that has been recently licensed for the management of skin and soft-tissue infections (13). It has excellent intrinsic activity against MRSA and methicillin-resistant CoNS but also vancomycin-resistant entero-

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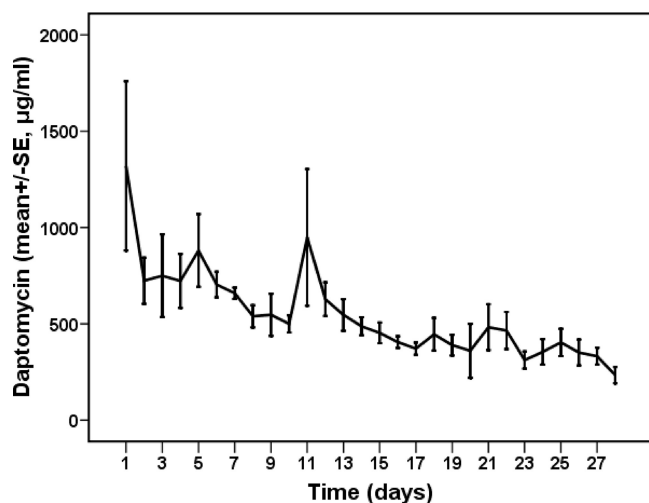


FIG. 1. Elution of daptomycin by Stimulan.

cocci (10). The MICs for 90% of the strains of MRSA, methicillin-resistant CoNS, and vancomycin-resistant enterococcal isolates tested are reported to be 0.78, 0.44, and 0.5 µg/ml, respectively (1, 4). Although the eluted daptomycin concentrations obtained with the system described here are much greater than the above-mentioned MICs for 90% of the strains tested, results should be interpreted with caution, since the kinetics of release apply to an in vitro system and not to in vivo conditions. It should, however, be underscored that eluted levels are considerably greater than the concentrations that are required to eliminate the growth of small-colony variants of *S. aureus* that are often implicated in chronic bone infections (1).

The estimated AUC of elution may be considered indirect evidence of the total amount of drug released (2). The AUC for daptomycin by the elution system presented here is much greater than the AUC required for the management of an experimental thigh infection with MRSA in mice (9).

Calcium sulfate has been applied as a carrier for daptomycin in two previous studies. The drug was admixed with CaSO₄ in pellet form. The total time of drug elution was 28 days, but the eluted concentrations were 10- to 100-fold lower than those achieved here (12). Even at a low rate of elution, the drug amounts released inhibited the growth of 10⁴ CFU of two different locally instilled isolates, one of *S. aureus* and another of *S. epidermidis* (14).

Although the effectiveness of daptomycin has not been evaluated in any prospective randomized clinical trial for the therapy of staphylococcal osteomyelitis, retrospective data favor its application for the management of such infections (8). These favorable clinical prospects, along with the successful in vitro elution of daptomycin from Stimulan reported in the present

study, support the application of the biodegradable system described here in experimental models of osteomyelitis.

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REFERENCES

- Baltch, A. L., W. J. Ritz, L. H. Bopp, P. Michelsen, and R. P. Smith. 2008. Activities of daptomycin and comparative antimicrobials, singly and in combination, against extracellular and intracellular *Staphylococcus aureus* and its stable small-colony variant in human monocyte-derived macrophages and in broth. *Antimicrob. Agents Chemother.* **52**:1829–1833.
- Cerretani, D., G. Giorgi, P. Fornara, L. Bocchi, L. Neri, R. Ceffa, F. Ghisellini, and M. A. Ritter. 2002. The in vitro elution characteristics of vancomycin combined with imipenem-cilastatin in acrylic bone-cements: a pharmacokinetic study. *J. Arthroplasty* **17**:619–626.
- Dandekar, P. K., P. R. Tessier, P. Williams, C. H. Nightingale, and D. P. Nikolau. 2003. Pharmacodynamic profile of daptomycin against *Enterococcus* species and methicillin-resistant *Staphylococcus aureus* in a murine thigh infection model. *J. Antimicrob. Chemother.* **52**:405–411.
- Hawkey, P. M. 2008. Pre-clinical experience with daptomycin. *J. Antimicrob. Chemother.* **62**(Suppl. 3):iii7–iii14.
- Kanellakopoulou, K., and E. J. Giamarellos-Bourboulis. 2000. Carrier systems for the local delivery of antibiotics in bone infection. *Drugs* **59**:1223–1232.
- Kanellakopoulou, K., N. Galanakis, E. J. Giamarellos-Bourboulis, et al. 2000. Treatment of experimental osteomyelitis caused by methicillin-resistant *Staphylococcus aureus* with a biodegradable system of lactic acid polymer releasing pefloxacin. *J. Antimicrob. Chemother.* **46**:311–314.
- Kanellakopoulou, K., G. C. Thivaos, M. Kolia, M., I. Dontas, L. Nakopoulou, E. Dounis, E. J. Giamarellos-Bourboulis, A. Andreopoulos, P. Karagiannakos, and H. Giamarellou. 2008. Local treatment of experimental *Pseudomonas aeruginosa* osteomyelitis with a biodegradable dilactide polymer releasing ciprofloxacin. *Antimicrob. Agents Chemother.* **52**:2335–2339.
- Lamp, K. C., L. V. Friedrich, L. Mendez-Vigo, and R. Russo. 2007. Clinical experience with daptomycin for the treatment of patients with osteomyelitis. *Am. J. Med.* **120**(10 Suppl. 1):S13–S20.
- LaPlante, K. L., S. N. Leonard, D. R. Andes, W. A. Craig, and M. J. Rybak. 2008. Activities of clindamycin, daptomycin, doxycycline, linezolid, trimethoprim-sulfamethoxazole, and vancomycin against community-associated methicillin-resistant *Staphylococcus aureus* with inducible clindamycin resistance in murine thigh infection and in vitro pharmacodynamic models. *Antimicrob. Agents Chemother.* **52**:2156–2162.
- Malli, E., I. Spiliopoulou, F. Kolonitsiou, D. Klapsa, E. Giannitsioti, K. Pantelidi, A. Pratti, M. Panopoulou, S. Grapsa, E. Alepopoulou, I. Neonakis, F. Frantziou, S. Alexiou-Daniel, D. Bakola, C. Koutsia-Carouzou, H. Malamou-Lada, L. Zerva, E. Vlahaki, S. Kartali-Ktenidou, E. D. Anastasiou, and E. Petinaki. 2008. In vitro activity of daptomycin against gram-positive cocci: the first multicentre study in Greece. *Int. J. Antimicrob. Agents* **32**:525–528.
- Panagopoulos, P., T. Tsaganos, D. Plachouras, D. P. Carrer, A. Papadopoulos, H. Giamarellou, and K. Kanellakopoulou. 2008. In vitro elution of moxifloxacin and fusidic acid by a synthetic crystalline semihydrate form of calcium sulphate (Stimulan). *Int. J. Antimicrob. Agents* **32**:485–487.
- Richelsoff, K., N. Webb, and W. Haggard. 2007. Elution behaviour of daptomycin-loaded calcium sulphate pellets: a preliminary study. *Clin. Orthop. Relat. Res.* **461**:68–73.
- Seaton, R. A. 2008. Daptomycin: rationale and role in the management of skin and soft tissue infections. *J. Antimicrob. Chemother.* **62**(Suppl. 3):iii15–iii23.
- Webb, N. D., J. D. McCanless, H. S. Courtney, J. D. Bumgardner, and W. O. Haggard. 2008. Daptomycin eluted from calcium sulphate appears effective against *Staphylococcus*. *Clin. Orthop. Relat. Res.* **466**:1383–1387.
- Wichelhaus, T. A., E. Dingeldein, M. Rauschmann, S. Kluge, R. Dieterich, R., V. Schäfer, and V. Brade. 2001. Elution characteristics of vancomycin, teicoplanin, gentamicin and clindamycin from calcium sulphate beads. *J. Antimicrob. Chemother.* **48**:117–119.
- Yamashita, Y., A. Uchida, T. Yamakawa, Y. Shinto, N. Araki, and K. Kato. 1998. Treatment of chronic osteomyelitis using calcium hydroxyapatite ceramic implants impregnated with antibiotic. *Int. Orthop.* **22**:247–251.